

WHAT IS CLAIMED IS:

1. A method for determining titre in a biological sample of known volume wherein said method comprises the steps of:
 - (a) concentrating microorganisms by i) adding a sample containing said microorganisms to an ultracentrifuge tube and ii) centrifuging said sample in said tube to concentrate said microorganisms, said ultracentrifuge tube comprising an upper region, a middle region and a lower region, wherein an inner diameter of said upper region is larger than an inner diameter of said lower region, wherein said upper region is separated from said lower region by said middle region having a decreasing diameter from said upper region toward said lower region and wherein said lower region has a closed bottom;
 - (b) removing fluid from above said lower region;
 - (c) overlaying remaining fluid with water or buffer less dense than fluid in said lower region;
 - (d) inserting a capillary tube with an open bottom end into said ultracentrifuge tube such that said open bottom end is above one or more microorganism bands;
 - (e) drawing fluid through said open bottom end of said capillary tube such that said fluid being drawn through said capillary tube forms a stream of fluid which exits said capillary tube and passes through a flow cell;
 - (f) adding water or buffer to said upper region of said ultracentrifuge tube as fluid is withdrawn in step (e) or as needed to maintain water or buffer above any viral band;
 - (g) moving said ultracentrifuge tube relative to said capillary tube such that said capillary tube moves into said lower region of said centrifuge tube and through any viral band of microorganisms;
 - (h) detecting microorganisms or a signal therefrom in said stream of fluid flowing through said flow cell to determine a number of microorganisms present; and
 - (i) calculating a titre from the determined number of microorganisms and known volume of said biological sample.
2. The ultracentrifuge tube of claim 1, wherein said middle region comprises one or more serrations.

3. The ultracentrifuge tube of claim 1, wherein said lower region has an inner diameter small enough to trap an air bubble between two layers of liquid such that the air bubble will keep said two layers of liquid separate so long as said centrifuge tube is at rest.
4. The method of claim 1, further comprising pumping fluid into a sheath around said stream of fluid exiting from said capillary tube thereby diluting said stream prior to passing through said flow cell.
5. The method of claim 4, wherein said sheath of fluid is pumped at a rate slower than the rate at which fluid passes through said flow cell.
6. The method of claim 5, wherein the flow of each fluid is controlled by gas pressure.
7. The method of claim 1, wherein said microorganisms are at a concentration in said capillary tube less than one-half their concentration in a band of microorganisms in said lower region of said centrifuge tube.
8. A method for determining titre in a biological sample of known volume wherein said method comprises the steps of:
 - (a) concentrating microorganisms by i) adding a sample containing said microorganisms to an ultracentrifuge tube and ii) centrifuging said sample in said tube to concentrate said microorganisms, said ultracentrifuge tube comprising an upper region, a middle region and a lower region wherein an inner diameter of said upper region is larger than an inner diameter of said middle region, wherein (i) an inner diameter of said middle region is larger than an inner diameter of said lower region or (ii) the inner diameter of said middle region is the same as the inner diameter of said lower region, wherein that inner diameter is small enough to trap an air bubble between two layers of aqueous liquid such that the air bubble will keep said two layers of aqueous liquid separate so long as said centrifuge tube is at rest, and wherein said lower region has a closed bottom;
 - (b) removing fluid from above said lower region;
 - (c) overlaying remaining fluid with water or buffer less dense than fluid in said lower region;

(d) inserting a capillary tube with an open bottom end into said ultracentrifuge tube such that said open bottom end is above one or more microorganism bands;

(e) drawing fluid through said open bottom end of said capillary tube such that said fluid being drawn through said capillary tube forms a stream of fluid which exits said capillary tube and passes through a flow cell;

(f) adding water or buffer to said upper region of said ultracentrifuge tube as fluid is withdrawn in step (e) or as needed to maintain water or buffer above any viral band;

(g) moving said ultracentrifuge tube relative to said capillary tube such that said capillary tube moves into said lower region of said centrifuge tube and through any viral band of microorganisms;

(h) detecting microorganisms or a signal therefrom in said stream of fluid flowing through said flow cell to determine a number of microorganisms present; and

(i) calculating a titre from the determined number of microorganisms and known volume of said biological sample.

9. The method of claim 8, further comprising pumping fluid into a sheath around said stream of fluid exiting from said capillary tube thereby diluting said stream prior to passing through said flow cell.
10. The method of claim 9, wherein said sheath of fluid is pumped at a rate slower than the rate at which fluid passes through said flow cell.
11. The method of claim 10, wherein the flow of each fluid is controlled by gas pressure.
12. The method of claim 8, wherein said microorganisms are at a concentration in said capillary tube less than one-half their concentration in a band of microorganisms in said lower region of said centrifuge tube.
13. A method of determining whether a homogeneous population of unknown microorganisms present in a biological sample comprises a known microorganism previously characterized by determination of a mass spectrum of proteins for said known microorganism, wherein said method comprises the steps of:

(a) concentrating microorganisms by i) adding a sample containing said microorganisms to an ultracentrifuge tube and ii) centrifuging said sample in said tube to concentrate said microorganisms, said ultracentrifuge tube comprising an upper region, a middle region and a lower region, wherein an inner diameter of said upper region is larger than an inner diameter of said lower region, wherein said upper region is separated from said lower region by said middle region having a decreasing diameter from said upper region toward said lower region and wherein said lower region has a closed bottom;

(b) recovering said unknown microorganisms in a concentrated form;

(c) subjecting said unknown microorganisms to mass spectrometry to measure the masses of individual proteins from said unknown microorganisms;

(d) determining a mass spectrum of said proteins from said unknown microorganisms; and

(e) comparing said mass spectrum of said proteins from said unknown microorganisms with mass spectra of proteins of known microorganisms;

wherein if said mass spectrum of said proteins from said unknown microorganisms is identical to one of said mass spectra of proteins from known microorganisms then said unknown microorganisms in said biological sample comprise the known microorganism having the one of said mass spectra of proteins to which said mass spectrum of said proteins from said unknown microorganisms is identical.

14. The ultracentrifuge tube of claim 13, wherein said middle region comprises one or more serrations.
15. The ultracentrifuge tube of claim 13, wherein said lower region has an inner diameter small enough to trap an air bubble between two layers of liquid such that the air bubble will keep said two layers of liquid separate so long as said centrifuge tube is at rest.
16. The method of claim 13, wherein said mass spectrometry is matrix assisted laser desorption ionization time of flight mass spectrometry.
17. The method of claim 13, wherein said mass spectrometry is electrospray mass spectrometry.

18. The method of claim 13, wherein said proteins are enzymatically digested prior to said subjecting step (c).
19. A method of determining whether a homogeneous population of unknown microorganisms present in a biological sample comprises a known microorganism previously characterized by determination of a mass spectrum of proteins for said known microorganism, wherein said method comprises the steps of:
 - (a) concentrating microorganisms by i) adding a sample containing said microorganisms to an ultracentrifuge tube and ii) centrifuging said sample in said tube to concentrate said microorganisms, said ultracentrifuge tube comprising an upper region, a middle region and a lower region wherein an inner diameter of said upper region is larger than an inner diameter of said middle region, wherein (i) an inner diameter of said middle region is larger than an inner diameter of said lower region or (ii) the inner diameter of said middle region is the same as the inner diameter of said lower region, wherein that inner diameter is small enough to trap an air bubble between two layers of aqueous liquid such that the air bubble will keep said two layers of aqueous liquid separate so long as said centrifuge tube is at rest, and wherein said lower region has a closed bottom;
 - (b) recovering said unknown microorganisms in a concentrated form;
 - (c) subjecting said unknown microorganisms to mass spectrometry to measure the masses of individual proteins from said unknown microorganisms;
 - (d) determining a mass spectrum of said proteins from said unknown microorganisms; and
 - (e) comparing said mass spectrum of said proteins from said unknown microorganisms with mass spectra of proteins of known microorganisms;

wherein if said mass spectrum of said proteins from said unknown microorganisms is identical to one of said mass spectra of proteins from known microorganisms then said unknown microorganisms in said biological sample comprise the known microorganism having the one of said mass spectra of proteins to which said mass spectrum of said proteins from said unknown microorganisms is identical.
20. The method of claim 19, wherein said mass spectrometry is matrix assisted laser desorption ionization time of flight mass spectrometry.

21. The method of claim 19, wherein said mass spectrometry is electrospray mass spectrometry.
22. The method of claim 19, wherein said proteins are enzymatically digested prior to said subjecting step (c).
23. A method of separating layers in an ultracentrifuge tube prior to centrifugation wherein fluid in said ultracentrifuge tube comprises a first dense layer and a second less-dense layer, wherein said method comprises the steps of:
 - (a) inserting said first dense layer into said tube;
 - (b) inserting into said centrifuge tube a means for separating the first and second layers prior to inserting said second less-dense layer into said tube; and
 - (c) inserting said second less-dense layer into said tube above said means such that said first dense layer is separated from said second less-dense layer by said means;wherein said ultracentrifuge tube comprising an upper region, a middle region and a lower region, wherein an inner diameter of said upper region is larger than an inner diameter of said lower region, wherein said upper region is separated from said lower region by said middle region having a decreasing diameter from said upper region toward said lower region and wherein said lower region has a closed bottom.
24. The ultracentrifuge tube of claim 23, wherein said middle region comprises one or more serrations.
25. The ultracentrifuge tube of claim 23, wherein said lower region has an inner diameter small enough to trap an air bubble between two layers of liquid such that the air bubble will keep said two layers of liquid separate so long as said centrifuge tube is at rest.
26. The method of claim 23, wherein said means for separating said layers is an air bubble.
27. The method of claim 23, wherein said means for separating said layers is a porous disk and said porous disk is inserted on top of said first layer.

28. The method of claim 27, wherein said disk is one which will float during centrifugation to a region above said second less-dense layer, thereby allowing said first dense layer to contact said second less-dense layer.
29. The method of claim 27, wherein said disk is made of sintered polyethylene or polypropylene.
30. A method of separating layers in an ultracentrifuge tube prior to centrifugation wherein fluid in said ultracentrifuge tube comprises a first dense layer and a second less-dense layer, wherein said method comprises the steps of:
- (a) inserting said first dense layer into said tube;
 - (b) inserting into said centrifuge tube a means for separating the first and second layers prior to inserting said second less-dense layer into said tube; and
 - (c) inserting said second less-dense layer into said tube above said means such that said first dense layer is separated from said second less-dense layer by said means;
- wherein said ultracentrifuge tube comprising an upper region, a middle region and a lower region wherein an inner diameter of said upper region is larger than an inner diameter of said middle region, wherein (i) an inner diameter of said middle region is larger than an inner diameter of said lower region or (ii) the inner diameter of said middle region is the same as the inner diameter of said lower region, wherein that inner diameter is small enough to trap an air bubble between two layers of aqueous liquid such that the air bubble will keep said two layers of aqueous liquid separate so long as said centrifuge tube is at rest, and wherein said lower region has a closed bottom.
31. The method of claim 30, wherein said means for separating said layers is an air bubble.
32. The method of claim 30, wherein said means for separating said layers is a porous disk and said porous disk is inserted on top of said first layer.
33. The method of claim 32, wherein said disk is one which will float during centrifugation to a region above said second less-dense layer, thereby allowing said first dense layer to contact said second less-dense layer.

34. The method of claim 32, wherein said disk is made of sintered polyethylene or polypropylene.

2025-10-27 10:54:54